Are Some Cases of Autism Actually Subclinical, Congenital Attenuated Rubella Syndrome?

A Collection of Data and Questions for Consideration and Review by the IOM Immunization Safety Review Committee during the February 9, 2004 Meeting on the Topic of Vaccines and Autism

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Introduction: A Link Between Maternal Rubella-Susceptibility & Autism Diagnoses in Offspring

The 36-year-old female author of this paper knows exactly three women who have a child that has been diagnosed with autism spectrum disorder [ASD]. One of these women grew up in Texas, one in Iowa and one in the Carolinas. An interesting thing these women have in common, in addition to being in their mid-30s and having ASD in their families, is that all three were found to be rubella-susceptible during their routine prenatal care and all three required post-partum rubella shots after having their children that would later be diagnosed on the autism spectrum.

These women are not alone. There are other moms with autistic children who either failed to seroconvert to a previous rubella vaccination and/or who were rubella susceptible during their pregnancies with their autistic children.

F. Edward Yazbak, MD, FAAP, found a link between mothers’ rubella susceptibility in pregnancy and their having children with autism spectrum disorders. He described 60 previously vaccinated women who were considered “rubella susceptible” during their routine prenatal OB/GYN visits. All of these women were revaccinated in the post partum period due to their not having antibodies to rubella. Each of these women could be described as having failed to seroconvert to an previous vaccination or having rubella immunity that declined over time.

According to Yazbak, among these 60 women, “45 have children diagnosed with autistic spectrum disorder [ASD], another ten women have children with autistic symptoms, ADD/ADHD or other developmental delays; and four women have children with other health problems, mostly immunologic.” (1)

Adding the three moms I know to those from this study, that’s 58 out of 63 or a 92% hit rate of non-seroconverters to rubella vaccine or those with waning immunity having children with autism spectrum disorders, autistic symptoms, ADD/ADHD or other developmental delays. Adding in the children with other, mostly immunologic health problems, the rate of health problems in offspring increases to 98%.

**In this group of 63 women who were rubella susceptible in pregnancy:**

- 76% have children diagnosed with autism spectrum disorders
- 92% have children with ASD, autistic symptoms, ADD/ADHD, developmental delays
- 98% have children with neurological and/or immunologic problems

Yazbak suggests the resultant ASD in offspring is due to an immune system problem in the mother which passed on to the child so that later when the child was vaccinated with MMR, autism was induced.

This paper introduces an alternate explanation to what appears to be an apparent link between the rubella vaccine’s failure to produce seroconversion and sufficient immunity in some females and their having a child or multiple children with autism spectrum disorders.
Overview of Persistent Rubella Infection and Autism Induction in Pregnancy Idea

The main idea proposed in this paper is that autism is induced during pregnancy when a persistent maternal infection with attenuated, vaccine strain rubella reactivates and infects the embryo or fetus developing in utero. After vaccination, the virus persists in vivo producing no clinical symptoms of rubella in affected females. These women do not know it but they are serving as hosts to the virus. When the host female becomes pregnant, the persistent attenuated virus is able to activate and replicate due to the natural suppression of the immune system that occurs in pregnancy. When the virus becomes active it can infect the developing embryo or fetus at critical times during the development of the central nervous system. The injuries sustained to the developing embryo or fetus manifest during the first few years of life as autism spectrum disorders. The symptoms range from no noticeable symptoms at birth to infantile autism to late or regressive autism in the first few years of life. Other neurologic and immunologic symptoms also develop over time as congenital infection with attenuated rubella, like wild type congenital rubella, produces new symptoms that continue to manifest slowly over many years and even decades. The injuries sustained in utero due to the persistent vaccine strain virus are not as severe or as noticeable at birth as those sustained with wild type rubella infection in pregnancy. The reason for this is that the vaccine-strain rubella virus is not as potent and has been weakened or “attenuated.” The persistent infection scenario would result in affected females having a child or multiple children with autism spectrum disorders as the mother unknowingly is serving as a host to the virus, and the virus can reactivate and replicate during each pregnancy when the immune system is suppressed.

This scenario provides a possible refutation for autism twin studies which have previously suggested a genetic basis given that identical twins are likely to produce 2 autistic children and fraternal twins more often produce only 1 autistic child. Mother-to-child virus transmission studies done for other viruses can be used for comparative purposes. Such studies typically show that when there is one placenta (i.e. identical or monozygotic twins) there is a greater likelihood that both twins are infected with the virus. When there are two placentas (i.e. fraternal or dizygotic twins) there is less likelihood that both children are infected.

A few ideas and questions are warranted on WHY some females may unknowingly be serving as hosts to a persistent rubella infection and why the RA27/3 vaccine strain of rubella virus might be particularly well adapted to persist undetected in females.

Why Might Vaccine-Strain Rubella Virus Persist in Some Females?

Information provided by the FDA in Appendix A on the history of the development of rubella vaccines used in the US prompts many questions about the safety of RA27/3 strain of rubella vaccine and its use in females. Per the FDA, the rubella virus used in the vaccine originated from a fetus that was aborted in 1964. It was isolated in and recovered from fetal tissue. It was attenuated through 25 passages through human fetal cells, and it is grown in human fetal cells.

Questions about RA27/3 Rubella Vaccine Origin:

The original isolated virus entered the reproductive tract of the 25-year-old mother, it crossed the placenta and it infected her fetus. Did the virus succeed in crossing the placenta by effectively mimicking the placenta’s molecular structures? Once inside the reproductive tract, did the virus “learn” and mimic structures or epitopes specific to the uterus or a fetus? Did this particular virus have success in infecting the fetus because it was well-adept at figuring out many cellular signaling pathways in the female reproductive tract? Is the original virus selected for
reproduction and vaccine development potentially hazardous to females and their ability to have a normal, healthy child?

**Questions about RA27/3 Attenuation Process:**
Does 25 serial passages of live virus through fetal cell cultures increase the probability or possibility for molecular mimicry of human molecular epitopes to occur? Does 25 passages in human cells increase the opportunity for the live virus to decode human cellular pathways? Is the live RA27/3 virus truly “attenuated” or does it have an enhanced knowledge of how to manipulate pathways and survive undetected in a human host? Is the RA27/3 attenuated rubella virus well suited to persist in the female reproductive tract? Applying 2004 scientific knowledge and scrutiny to the attenuation process that was used in 1960’s for the RA27/3 virus, is this method of attenuation still considered safe?

**Questions about RA27/3 Strain Growth & Propagation:**
With growth of the live virus in fetal cell cultures is the live virus given yet another chance to decode human cellular pathways and molecular structures? Other vaccines that are grown in human tissue contain DNA and human protein. (2) If a post-pubertal female is given a live rubella virus vaccine containing ‘other human” DNA and human protein at the time of ovulation or during the luteal phase of her menstrual cycle, is her immune system in a receptive state allowing foreign DNA, foreign human materials and anything else foreign that presents simultaneously to be tolerated? As ovulation is a natural time for the female immune system to be receptive and tolerant is this a time that should be avoided when injecting vaccines containing live viruses combined with human DNA and human protein?

**Note:** See comment box on page 15 for the origin of the author’s concerns about persistent vaccine strain rubella virus.

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**What Data in the Literature & What Facts May Support these Ideas?**

**Data Point 1: Failure Rate of Rubella Vaccines**
The package insert for rubella vaccine states that 1-5% of vaccinees fail to seroconvert and develop antibodies against rubella. (3) It is well known that some individuals get repeated doses of rubella containing vaccines and they continue to fail to produce antibodies against rubella.

**Question:** What happens to the live rubella virus that is administered in a vaccine if an immune response is not activated and antibodies are not produced? Where does the live virus go? Might the virus enter the host, avoid detection through methods proposed previously and then persist undetected so that the vaccinee serves as host to live attenuated rubella virus? Is there any correlation between the 1-5% failure rate of rubella vaccines and the 1-in-150-250 children current rate of autism?

**Data Point 2: Chronic Rubella Viremia Post Vaccination**
Tingle, Chantler, et al published several reports in the 1980s about their finding vaccine strain rubella virus persisting in the synovial fluid and joints of arthritic patients as long as several years post vaccination. (4) The 1991 IOM report on Adverse Side Effects of Pertussis and Rubella Vaccines states, “in any event, it is not clear at this time whether patients who develop arthritis,
acute or persistent, after rubella vaccination have a specific immune system defect that prevents their systems from clearing the virus normally.” (5)

**Question:** Was consideration given to potential design flaws in the vaccine? Was consideration given to the potential long-term side effects that a persistent rubella infection may have on females’ reproductive health? Was consideration given to possible injuries that could be sustained to future offspring if reactivation of the persistent rubella virus occurred during pregnancy causing intrauterine infection?

**Data Point 3: Persistent Rubella Infection Feasible**

Cunningham, AL, studied persistent rubella virus infection of synovial cells cultured in vitro and concluded, “that persistent infection of synovial tissue in vivo is a feasible explanation for the presence of rubella virus in peripheral joints of patients with chronic arthritis.” (6)

**Data Point 4: Persistent Rubella Virus Reactivates when Cell-Mediated Immunity is Suppressed**

Bosma, et al, reported in 1998 that “persistence of rubella virus is also suggested by our previous studies, which demonstrated that rubella virus-specific IgM may persist for up to 4 years following both natural RV infection and vaccination with HPV77.DE5.” While their studies failed to confirm that rubella virus is associated with chronic inflammatory joint disease, “they do suggest that rubella virus may persist within a joint and be reactivated when cell-mediated immunity is suppressed.” (7)

It should be noted that the Bosma study reported on 79 vaccinees and the evaluation of synovial fluid samples. Of the 79 subjects in the study only 23, or fewer than 30% of those in the study were females.

**Question:** Shouldn’t any follow-up studies done on persistent rubella infection be focused on women given the potential risk of intrauterine infection in pregnancy?

**Data Point 5: Pregnancy Induces Maternal Immune System Suppression**

It is well accepted that the maternal immune system is suppressed in pregnancy to allow the embryo which is comprised of foreign DNA to survive maternal immune attack.

**Question:** If attenuated rubella virus can persist in vivo as studies have shown, then can the virus be reactivated in a maternal host during immunosuppression in early pregnancy? If the virus replicates, can it infect the embryo or fetus and what are the outcomes to the child? Can a flare of a persistent subclinical, attenuated rubella infection at the right time in the fetus’ development cause damage to the developing central nervous system and produce a child that is born autistic but whose symptoms are not noticeable at birth but manifest over the 1st few years of life?

**Data Point 6: The Embryological Origin of Autism**

Rodier, et al, have reported on the embryological origin of autism, noting in particular the craniofacial abnormalities that are often seen with autism and that such abnormalities could only occur during the time of neural tube closure in development in utero. She also reported, “the craniofacial symptoms have been ignored in the autism literature, because they seem trivial in comparison to the disabling behavioral symptoms, but they speak directly to the embryological origin of the disorder.” (8)
Data Point 7: Timing of Manifestations of Symptoms of Congenital Rubella
Wild type rubella infection during the 1st trimester of pregnancy is a known cause of autism. Typically, with wild type rubella infection autism is one of many noticeable and severe symptoms that manifest from the congenital infection. Additional severe and very noticeable clinical symptoms that may be apparent at birth include cataracts, deafness, insulin-dependent diabetes mellitus. Late-onset manifestations include chronic immunologic dysfunction and vascular and central nervous system disease. (9) O’Neill’s report on long-term outcomes of maternally transmitted rubella “confirms that a broad spectrum of fetal injury may result from intrauterine infection and that both persistent and delayed-onset effects may continue or occur as late as 30 years after original infection. Many factors contribute to the varied outcome of prenatal infection, the 2 most important being the presence of maternal immunity during early gestation and the stage of gestation during which fetal exposure occurs in a nonimmune mother.” (10)

Question: If infection with wild type rubella in pregnancy produces autism PLUS profound, severe and immediately noticeable symptoms at birth, then can maternal infection with attenuated rubella produce autism MINUS profound, severe and immediately noticeable symptoms at birth?

Data Point 8: Timing of Manifestations of Symptoms of Autism
It is documented in the literature that the most noticeable behavioral symptoms associated with autism typically appear in the 2nd year of life. Those supportive of the embryologic origin of autism note that in family videos there was evidence of subtle autistic behaviors during the 1st year of life. Typically these observations are used to support the idea that symptoms of autism appeared prior to MMR vaccine and hence MMR vaccine in the child is not the root cause of autism.

The ideas in this paper support that it is not always MMR that induces autism in the child but rather vaccine strain persistent rubella in the mother that is producing instances of autism with an underlying embryologic cause. As the ideas herein suggest that vaccines which use human cell cultures in manufacturing and/or which contain human DNA have the capacity to produce persistent vaccine strain viral infections, MMR is a candidate root cause agent of persistent infections in some children post vaccination which may produce subsequent immunological dysfunction and health problems.

Data Point 9: Repeated Rubella Vaccine Failures & No Boost with Attenuated Strain Challenge
Just, et al conducted a 15-year post rubella vaccination follow up study on young women who were seronegative for rubella antibody pre-vaccination and who were vaccinated at 15-25 years of age against rubella with the Cendehill vaccine strain. Among 319 women retested for rubella immunity 15 years later, 3 vaccinees (or nearly 1%) were seronegative by all three tests used in the study to determine rubella immunity. These three women also showed no booster response after challenge with the vaccine strain. (11)

Question: Were these women failing to respond to the rubella virus because they were already tolerating and serving as hosts to that strain of virus? Is there something about their genetic makeup and the Cendehill strain’s design that makes these individuals and this strain of vaccine incompatible? Why would they continue not to boost, and what was happening to the vaccine virus each time it was injected? Can today’s instances of autism be traced back to a particular strain of rubella vaccine that was used prior to RA27/3, or given the autism “explosion” that is occurring now, 23 years after RA27/3 became the only rubella strain used in the US, is RA27/3 rubella vaccine the more likely culprit?
Data Point 10: Subclinical Rubella in “Immune” Mothers Can Still Infect Fetus

Dr. Theresa Tam’s 1999 report, The Many Expressions of Congenital Rubella states: “Maternal infection without a rash in pregnancy can still lead to fetal disease. Congenital rubella after maternal re-infection has occasionally been documented, therefore a maternal history of rubella immunity before pregnancy must not preclude the investigation of an infant with compatible symptoms.” (9) Aboudy et al reported that subclinical rubella infection can occur in previously vaccinated women and it can result in transmission of the virus to the fetus in pregnancy if titres are low. (12) Saule, et al reported that a mother with confirmed rubella immunity after previous vaccination had a son with congenital rubella infection. The report stated, “intrauterine transmission must have occurred after maternal reinfection during pregnancy. Prenatal diagnosis of rubella embryopathy with serological methods after subclinical maternal reinfection is nearly impossible.” (13)

Data Point 11: “False Positive” Rubella IgM Responses in Pregnancy

According to Laboratory Corporation of America, false-positive rubella IgM responses have been reported in pregnant women. Other false-positive rubella IgM responses have been reported following mononucleosis, parvovirus B19 infections, and possibly other herpes-type viral infections. These reactions are usually accompanied by false-positive reactions to other viruses (eg, CMV and measles). (14)

Question: If a person is host to a persistent, attenuated rubella infection which flares during a time of immunosuppression such as pregnancy, and if the infection is subclinical, might mistakes be common in interpreting lab results as false positives?

Data Point 12: Microbes that are Supposed to be “Attenuated” Sometimes are Not

Oaklandtribune.com recently reported that scientists at UC Berkeley, in attempting to render tuberculosis bacteria harmless, actually created a more virulent and lethal bacteria. “In trying to make tuberculosis less infectious, Berkeley scientists created a superbug that killed every lab mouse it touched…they disabled a collection of genes associated with the bacteria’s invasion of healthy cells [and] they ended up with one of the world’s few “hypervirulent” organisms.” (15)

Question: Is the “attenuated” rubella virus used in vaccines really attenuated given its origin and the development methods used in manufacturing? Because the rubella virus used in vaccines (RA27/3 strain) was derived from an infected human fetus, and because that isolated virus was passaged 25 times through human fetus cell cultures and because that “seemingly weakened” virus is grown in human fetus cell cultures (WI-38), is the vaccine strain virus truly attenuated? Or has the attenuated virus been given many opportunities to mimic molecular structures and decode cellular signaling pathways so that it is well suited to persist undetected particularly in females and possibly even in female reproductive tracts?

Data Point 12: The Y-Chromosome and the Male Factor Ratio in Autism

Ingemarsson’s report on gender aspects of preterm birth cites “studies in recent years indicate that sex differentiation begins at conception. The SRY gene on the Y-chromosome is already transcribed at the 2-cell stage and triggers growth acceleration in the XY embryos. This accelerated growth is believed to be important for the male embryo as it allows complete testicular differentiation before the levels of oestrogenic hormones become too high as pregnancy progresses.” (16)
Question: Could male embryos trigger a greater initial maternal immunosuppressive state than female embryos due to the need for a mother’s immune system to tolerate a blatantly “non-self” and “foreign” Y-chromosome? In the paradigm of persistent rubella infection in the mother and reactivation of the virus during pregnancy, could a “more suppressed” maternal immune system that is supporting rapid growth of an XY embryo provide greater vulnerability of male embryos to be exposed to reactivated attenuated rubella prior to the time of neural tube closure? Might this be a possible explanation for the male factor ratio in autism?

Data Point 13: Female and Male Immune Systems Respond Differently to Rubella Vaccine
Research has shown that there are differences in how adolescent males and females respond to RA27/3 rubella containing vaccines. Mitchell’s studies on immune responses after re-immunization suggest “that there are hormonal influences on rubella virus-specific immunity which might result in differential handling of rubella virus and, hence, may partially explain why females are more predisposed to adverse outcomes of rubella infection and immunisation. (17). Shohat, et al, studied sex differences with measles-containing vaccines and concluded, “Our findings demonstrate higher rates of adverse effects in females following vaccination with MMR vaccine, irrespective of the humoral response. This study emphasizes the need to consider possible gender differences when evaluating new vaccines.” (18)

Question: Given the maternal/placental/fetal host origin, and given the repeated and continual use of products of a female reproductive tract in manufacturing, should we be surprised that females respond differently than males to RA27/3 rubella containing vaccines?

Data Point 14: Anti-Rubella Virus IgG & Autoimmunity
Besson, D, et al, reported, “To support the hypothesis that demyelination was caused by anti-rubella virus IgG that recognized an MOG [myelin oligodendrocyte glycoprotein] epitope, we found that anti-rubella virus antibodies depleted MOG in a dose-dependent manner. Further evidence came from the demonstration that anti-RV and anti-MOG IgG colocalized on oligodendrocyte processes and that both revealed by Western blot a 28 kDa protein in CNS myelin, a molecular weight corresponding to MOG. These findings suggest that a virus such as rubella virus exhibiting molecular mimicry with MOG can trigger an autoimmune demyelination.” (19)

Question: Is further study on rubella vaccine strain viruses warranted given that rubella virus exhibiting molecular mimicry with human cell epitopes can trigger autoimmunity?

Data Point 15: Intrauterine Infection, Fetal Inflammatory Response & Neurodevelopment
Schendel, reporting for the National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, states: “Infections in pregnancy, including the most common congenital infections (TORCH: toxoplasmosis, rubella, cytomegalovirus, herpes simplex virus), are known causes of long-term neurodevelopmental disabilities.” “Intrauterine infection, especially subclinical infection of the kind associated with preterm birth, is under investigation as a cause of neurodevelopmental disability.” “These data suggest that factors related to the fetal inflammatory response, including cytokines, may be causal agents in brain damage and neurodevelopmental disability associated with intrauterine infection. We need to greatly improve both our understanding of and our ability to measure the relevant exposures related to infection and inflammation…and to investigate a broad range of neurodevelopmental outcomes as potential adverse effects of intrauterine infection.” (20).
Concluding Remarks & Requests

According to the Institute of Medicine website, the IOM functions as a private, non-government, nonprofit organization whose “mission is to serve as advisor to the nation to improve health,” and “to ensure scientifically informed analysis and independent guidance,” and to “provide unbiased, evidence-based, and authoritative information and advice concerning health and science policy to policy-makers, professionals, leaders in every sector of society, and the public at large.”

According to the FDA/CBER division, IOM, in its previous review of a potential association between MMR and autism, did not consider the possibility that autism may be a result of a long term side effect of rubella-containing vaccines in mothers of autistic children. IOM has not previously reviewed any potential association between rubella containing vaccines, their capacity to induce persistent infections in females, the potential harm that a chronic attenuated rubella infection might produce in the unborn, and whether or not such infection may induce autism in offspring.

The purpose of this paper is to urge IOM to advise FDA/CBER division and other appropriate groups in the public and private sector to research this scenario further based on the information herein and in particular the study of 63 rubella-susceptible women of whom 98% have children with ASD, autistic symptoms, ADD/ADHD and other immunologic problems.

Another purpose of this paper is to urge IOM to investigate further why vaccine strain viruses persist in some vaccinees. Using 2004 knowledge about molecular mimicry, cellular pathways, subcellular processes and genetics, and using 2004 diagnostic technologies, I hope IOM can offer the public at large answers on why vaccine viruses might persist, and who, from a genetic perspective, is more likely to have this occur. With this data, IOM can then make any needed recommendations on types of individuals for whom rubella containing vaccines may be contraindicated.

A third purpose of this paper is to urge IOM to advise FDA/CBER to institute a controlled and well-managed post-marketing surveillance program of vaccines. FDA/CBER and vaccine manufacturers should be required to conduct periodic, continually updated safety reviews of vaccine products that are mandated for public use. Consumers should not be expected to feel reassured by vaccine safety studies and citations that are 40 years old. Vaccines should be held to the highest safety standards on an ongoing basis.

A fourth purpose of this paper is to urge IOM to recommend immediate further study on if and how vaccines which use human fetus cell cultures in manufacturing might impair female fertility, induce pregnancy complications and produce children with autism, neurological and immunological abnormalities. According to the March of Dimes, 1 in 8 babies born in the US today is born premature. Kucukoduk S, et al, “reached the conclusion that Low birth weight may be related to asymptomatic intrauterine rubella infection.” (19) According to the Royal College of Obstetrics, 1 in 100 women experience infertility in the form of recurring spontaneous abortions. Autism is occurring today at a rate of 1-in-150 children. Are vaccines which use human fetus cells in manufacturing playing a role in these tragic statistics?
A final data point and questions:

Of Yazbak’s 60 rubella-susceptible women who were immunized in the post partum period, “six of the mothers in this study, who had no prior obstetrical difficulties, reported having miscarriages after receiving the postpartum vaccination. There were also two cases of difficult pregnancies, and one case in which a woman carrying twins lost one of the children.” (1)

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<th>After RA27/3 rubella immunization post partum, among the 60 women in Yazbak’s study,</th>
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<td>10% experienced subsequent miscarriages</td>
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<td>3% experienced subsequent pregnancy complications</td>
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<td>1% experienced a twin pregnancy where one child was lost</td>
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Questions:
Can live virus vaccines which use human fetus cells in manufacturing induce females to have an immune response to a pregnancy?

Could foreign human DNA in vaccines induce an antibody response or cellular immunity to “foreign” DNA so that immune mediated pregnancy complications and miscarriages result? It is known that some females in a non-pregnant state have labs that are negative for anti-nuclear antibodies, but who when pregnant develop positive labs for anti-nuclear antibodies.

If natural killer cells and tumor necrosis factor [TNF-a] can terminate a pregnancy and cause complications resulting in pre-term birth, then can these cytokines and an inflammatory maternal response to a pregnancy cause autism to be induced through damage to the developing central nervous system? In other words could autism be a result of vaccine induced maternal immunity to an embryo or fetus?
References:


(2) Varivax, the varicella vaccine which has been available in the US since 1995, uses MRC-5 fetal cells as a growth medium. The package insert says that “each dose contains…residual components of MRC-5 [fetal] cells including DNA and protein.” Since the product’s introduction, there have been documented reports of spontaneous abortions in pregnant women and congenital anomalies. The manufacturer has set up a pregnancy registry and together with the FDA, they have taken steps to educate healthcare providers and consumers that Varivax is contraindicated in pregnancy.

(3) Excerpts from package inserts for rubella-containing vaccines Meruvax II and MMRII state: “a small percentage (1-5%) of vaccinees may fail to seroconvert after the primary dose.” “Meruvax II has not been evaluated for carcinogenic or mutagenic potential, or potential to impair fertility.” “Animal reproduction studies have not been conducted with Meruvax II. It is also not known whether Meruvax II can cause harm when administered to a pregnant woman or can affect reproduction capacity. There is evidence suggesting transmission of rubella vaccine viruses to products of conception. Therefore, rubella vaccine should not be administered to pregnant females.”


Chantler, JK, Tingle, A J, Petty R E, NEW ENGLAND JOURNAL OF MEDICINE , 313(18):1117-1123 1985, Persistent rubella virus infection associated with chronic arthritis in children


http://www.cps.ca/english/CPSP/Resources/Rcongenitalrubella.htm


http://www.labcorp.com/datasets/labcorp/html/chapter/mono/se016100.htm

(15) Oaklandtribune.com, Berkeley scientists create tuberculosis 'superbug' Article Last Updated: Saturday, December 27, 2003 - 2:58:58 AM PST.


Appendix A:

Information provided from CBER/FDA on the origin and history of rubella and measles vaccines

Note: The following information about Rubella and Measles vaccines was provided in a written personal communication from the FDA.

1. Background information on the history of rubella vaccines:

Two rubella vaccine strains have been licensed in the US, HPV77 and RA27/3. Merck was the first to receive a license for the production and distribution of HPV77 rubella vaccine in 1969. The first generation combination MMR (measles-mumps-rubella) vaccine containing HPV77 rubella strain was licensed in 1971. Wellcome Research Laboratories was licensed for the production of RA27/3 rubella vaccine in 1971. Subsequently, Merck was also licensed for the production of monovalent rubella vaccine derived from RA27/3 in 1978. Merck then formulated a second-generation combination vaccine, MMRII®, containing RA27/3 rubella vaccine virus, and this trivalent vaccine was licensed in 1979.

The HPV77 strain was originally isolated from an 8 year old boy with typical symptoms of rubella during an epidemic in Pennsylvania in 1962. This strain was attenuated by serial passage in grivet monkey kidney cells, followed by several passages in duck embryo cell cultures. Dr. Edward Buynak described the passage history of this strain in detail in reference 1. The vaccine was produced in duck embryo cells. This vaccine is no longer licensed for use in the US.

The strain of rubella vaccine currently licensed in the US, RA27/3, was isolated in 1964 from an aborted, rubella-infected human fetus following a documented infection in a 25-year-old mother. The rubella virus was recovered from kidney tissue cultured from this fetus, and the rubella-infected fetal kidney fibroblast cells were subsequently sub-cultured four times. Supernatant fluid from the fourth kidney cell passage (not the infected cells) contained rubella virus, and this liquid was used to infect WI-38 human lung diploid fibroblast cells. Virus recovered in the infected supernatant fluid from the initial passage in WI-38 cells was subsequently attenuated by 25 additional serial passages in WI-38 cells (2). The US licensed RA27/3 rubella vaccine manufactured by Merck is grown in WI-38 cells.

We would also like to clarify the derivation of the cells used for the production of rubella vaccine. WI-38 cells were originally obtained from a female fetus aborted at 3 months gestation from a healthy mother (3). Please note that 3 of the 25 passages of RA27/3 in WI-38 cells represent virus harvested from an infection using a terminal dilution of the inoculum (4). This means that supernatant containing virus was collected and then serially diluted 10-fold. Each 10-fold dilution was used to inoculate separate cultures of WI-38 cell monolayers. Only supernatants from cells that grew virus at the highest (most dilute) of the serial 10-fold dilutions were collected and further cultured. This serial
passage of the supernatant fluid and three terminal dilution steps virtually eliminates the possibility of any carryover of cells, cellular antigens, cytokines or any other alleged toxic chemicals from the original rubella infected fetus. The vaccine is grown in WI-38 cells but does not contain intact cells.


There is a nice summary describing the origin and development of measles vaccine strains in the textbook *Vaccines*, edited by Stanley Plotkin and Edward Mortimer. In the second edition, this information is in Chapter 9, Measles Vaccines, pages 237-240 and in Figure 9-2.

Names associated with measles vaccines:

**Edmonston:** refers to David Edmonston, who was a 13 year old boy residing at boarding school in Southboro, Massachusetts during a measles outbreak in 1954. Virus was isolated from his blood and subsequently serially passaged to develop measles vaccine strains. He is alive and well and living in Potomac, Maryland. Details of the original isolation may be found in the publication by Enders and Peebles, Propagation in Tissue Culture of Cytopathic Agents from Patients with Measles, Proceedings of the Society of Experimental Biology and Medicine, 1954, pp277-286, see details of Case 3, D.E. This strain in subsequent publications is identified as the 749D strain.

**Enders:** refers to Dr. John Enders who isolated and attenuated the Edmonston strain of measles virus with the help of Drs. Peebles, Katz, Chang and Holloway to develop the Edmonston B strain of measles vaccine.

**Schwarz:** refers to Dr. Anton Schwarz who obtained Edmonston virus (after 24 passages in human kidney cells, 28 passages in human amnion cells, 6 passages in chick embryos and 12 passages in chick embryo fibroblast cells) from Dr. Enders and serially passaged it 85 times in chick embryo fibroblast cells (5 times at 37 C to generate a strain named “Edmonston A” followed by 80 passages at 32 C) to develop the Schwarz strain of measles vaccine which he named after himself. Details of the development of this strain are documented in his publication in Annales of Paediatrici, 1964, 202: 241-252.

**Hilleman:** refers to Dr. Maurice Hilleman who with colleagues at Merck serially passaged Edmonston B vaccine virus 40 additional times in chick embryo tissue cultures at 32 C to develop the Moraten (for more attenuated) strain of measles vaccine now also known as the Edmonston-Enders strain of vaccines virus.

Details of the development of **Edmonston B** vaccine may be found in the publication by Enders, Katz and Holloway, AJDC, 1962, 103: 335-340, “Development of Attenuated Measles Virus Vaccines”.

Details of the development of **Moraten** vaccine strain are in the publication by Hilleman et al, “Development and Evaluation of the Moraten Measles Virus Vaccine, JAMA, 1968, 206: 587-590.

David Edmonston, a 13 year old boy in private boarding school in Massachusetts was diagnosed as having measles during an outbreak at that school in February 1954. Virus was isolated from a sample of his blood taken at this time by Drs. Enders and Peebles in cultures of human embryonic kidney (HEK). Trypsin was used to produce stationary cultures and cells were grown in culture.
medium consisting of bovine amniotic fluid (90%), beef embryo extract (5%), horse serum (5%),
antibiotics, and phenol red. The Edmonston strain was carried through 24 passages in HEK cells.
Culture fluid from the 24th passage was used to inoculate cultures of human amnion cells (HA)
and virus was similarly serially passaged 28 times in human amnion cells. Fluid from the 28th
amnion cell passage was used to inoculate chick embryos (CE). After 6 transfers of the chick
embryo adapted virus, the virus was serially passaged in chick embryo cells (CEF) 13 times
(Edmonston HEK24, HA28, CE6, CEF 13). Dr. Enders continued to attenuate the virus using 6
additional passages in chick embryos and then 11 passages in chick embryo fibroblast cells using
medium 199 (to reduce the protein content of the vaccine) to produce the EDMONSTON B
VACCINE STRAIN.

Dr. Hilleman used lot 254 of the Edmonston B vaccine strain (HEK24, HA28, CE 6, CEF13,
CE6, CEF11 @36-37 C) and passaged this virus 40 additional times in CEF cultures at 32 C to
produce the MORATEN VACCINE STRAIN.

Dr. Schwarz received Edmonston virus from Dr. Enders after the following passages: HEK24,
HA28, CE6, CEF13 and he serially passaged the virus 5 times in chick embryo fibroblast cells at
35 C to create a vaccine designated Edmonton A. He subsequently passaged this virus 80
additional times in chick embryo fibroblast cells at 32 C. This strain is designated SCHWARZ
VACCINE STRAIN and it was marketed by Pittman-Moore Dow in the US but it is no longer
licensed in this country.
Appendix B:

Information provided by CBER/FDA in May 2003 in response to the safety questions posed about rubella vaccines

COMMENT: The following information about rubella vaccines was provided in a letter from the FDA-CBER division in response to long-term safety questions posed about rubella containing vaccines and vaccines which use human fetus cell cultures in the manufacturing process. The original concerns submitted to FDA/CBER did not inquire about the possible role of molecular mimicry and viral decoding of cellular signaling pathways. IOM is the first audience to review these concerns in that context. Excerpts from the FDA/CBER letter follow with author’s comments in text boxes.

3. Can rubella vaccine virus cause a persistent infection?

You have expressed a concern that rubella vaccine virus may persist for up to 15 years after immunization and that this persistent infection may be associated with a state of tolerance that would permit transmission of rubella vaccine virus to an unborn child.

COMMENT: the author’s concern about persistent vaccine-strain rubella virus first came from email communications with a reproductive health expert and scientists who work with women experiencing recurring spontaneous abortions. As the motivation to have a child is so very strong, infertile couples and those experiencing recurring miscarriages spend tens of thousands of their own dollars to try to conceive and correct their infertility problems. Pathological evaluation of tissue from miscarriages is conducted regularly. PCR testing on tissue obtained from D&C procedures after miscarriages has shown the presence of the live rubella virus as long as 15 years post vaccination.

Rubella virus in pregnancy is not only a known cause of autism, but also a known cause of miscarriages. The author was unable to find published reports to support these findings and concerns but remains confident in the source. A suggestion for IOM is to contact labs that are conducting studies on miscarriage tissue and do surveillance on the PCR detection of vaccine-strain rubella virus in the products of miscarriage.

You have hypothesized that if there were persistent maternal infection, infants could then be exposed to rubella vaccine virus in utero and might be susceptible to central nervous system infection. We are not aware of any published report that supports a claim of prolonged, persistent RA27/3 rubella vaccine virus infection in normal individuals, and we know of only a single case describing polymerase chain reaction (PCR) detection of RA27/3 genome for no more than 8 months following inadvertent immunization of an immune compromised 16-year-old individual with leukemia (6). Clinical studies performed in healthy rubella-susceptible individuals prior to licensure of RA27/3 and HPV77 vaccine strains showed that vaccine virus was excreted from the throat less frequently and for a shorter duration than wild type rubella virus, and the quantity of virus shed was very low. Nasopharyngeal excretion of RA27/3 vaccine virus was detected in almost 50% of vaccinees and occurred on days 9 through 17 after immunization and virus
was typically shed for only one or two days (7). Likewise, HPV77 vaccine was shed sporadically from 75% of vaccinees on days 7 through 21 days after immunization (8).

It is presumed that viremia must also be rather common since the virus has traveled from the site of inoculation to the throat. However, live vaccine virus has been recovered from blood rarely and briefly sometime between 7 to 11 days after immunization but was not detected thereafter (9). These findings are complemented by more recent work using fluorescent antibody cell sorter (FACS) analysis to assess viremia following experimental infection with wild type rubella virus or immunization with RA27/3 vaccine. Rubella antigens were expressed on 9-51% of monocytes from each of 4 volunteers experimentally challenged with wild type rubella on days 1-13 day after infection.

In contrast, rubella antigens were detected on only 1-12% of monocytes obtained from 4 of 5 volunteers immunized with RA27/3 vaccine. Vaccine virus-infected monocytes were only detected between days 5 and 12 after immunization, and the proportion of infected cells was much lower than that seen after infection with wild type virus (10). These data, while based on small numbers, are consistent with previous work showing that viremia after vaccination occurred at a very low level and was brief. We are aware of work from one laboratory at the University of British Columbia that published evidence that HPV77 vaccine virus may persist in lymphocytes of individuals with chronic arthritis for up to 6 years after immunization (11). However, other investigators have not been able to duplicate these findings, and attempts to identify individuals with persistent RA27/3 vaccine virus infection have only confirmed that virus does not persist in blood for prolonged periods of time (12).

4. Can rubella vaccine virus cause infection in utero?

Studies performed to assess risk of vaccine virus transmission to a fetus exposed through inadvertent immunization of a rubella-susceptible mother in early pregnancy indicated that this risk, while not zero, is extremely low (13, 14). “Transplacental passage of vaccine virus is evidently rare having occurred four times in 708 pregnancies (of rubella susceptible women) complicated by RA27/3 immunization. More important, there was no congenital rubella syndrome case demonstrated in more than 1000 pregnancies during which rubella vaccines were given.” (15). More recently, investigators in Germany described an infant born with documented RA27/3 vaccine virus infection following inadvertent immunization of the mother in early pregnancy. After birth, this infant shed virus for 8 months, and genome sequence comparisons revealed that the virus from the child was identical to RA27/3. Importantly, this infant did not have any gross or subtle signs of congenital rubella syndrome and remained in good health when last examined at age 3 years, suggesting that the vaccine strain does not cause teratogenic effects (16, 17).

5. Can rubella vaccine virus infect the central nervous system?

Additional evidence indicates that rubella vaccine strains do not infect the central nervous system. Because wild type rubella infection may cause encephalitis, the rubella vaccine strains licensed in the US, including RA27/3 and HPV77, were each evaluated by neurovirulence testing in rubella-susceptible monkeys (see publication listed in reference 1 for information on the testing of HPV-77 rubella vaccine). Monkeys received combined intracerebral, intraspinal and intramuscular injections of vaccine and were then evaluated clinically for 17-21 days prior to sacrifice. In these tests, there was no evidence of illness or paralysis in the monkeys inoculated with vaccine and no evidence of virus replication or
inflammation upon histological review of multiple sections of the brain and spinal cord. Encephalitis attributable to rubella vaccine virus infection has not been documented, and we are not aware of any report describing the recovery of RA27/3 or detection of RA27/3 genome in brain or spinal fluids of vaccinated individuals with central nervous system disease.

**COMMENT:** The evaluations on central nervous system damage cited are not reassuring. The studies evaluated monkeys post immunization for only 17-21 days and then the monkeys were killed and it was determined using 1960’s knowledge and technology that no central nervous system damage was done. All mandatory vaccines should be required by law to continually be scrutinized for safety given the fact that accepted truths in science change, knowledge advances and new enabling diagnostic technologies continue to emerge.

6. Can rubella vaccine induce immune tolerance?

You have also expressed concern that rubella vaccine virus induces tolerance in certain vaccinated individuals. Because tolerance is known to occur in some infants with congenital rubella infection due to wild type virus, it is reasonable to ask if tolerance occurs after vaccination. Challenge studies in vaccinated individuals provide the most direct evidence that rubella immunization does not induce tolerance. Several studies document four-fold or greater rises in rubella specific antibody following intranasal inoculation with virulent or live attenuated strains.

For example, Dr. Meyer reported seroconversion of 10 of 10 rubella-susceptible children at an orphanage exposed to a roommate with wild-type rubella infection; 3 vaccinated children with low pre-exposure rubella antibody titers had a booster antibody response after the same exposure, and 12 vaccinated children with high pre-exposure rubella antibody titers did not boost because they already had sufficient antibody to neutralize the infecting dose (18). Similarly, 3 of 5 girls given an intranasal challenge with virulent rubella virus 8 to 12 months after HPV77 immunization experienced an anamnestic antibody rise. Wilkens et al also reported similar results for 8 HPV77 immunized children challenged with wild rubella virus intranasally 180 days after immunization. In his study, four of 8 vaccinees showed a four-fold or greater rise in rubella antibody (19) and one other vaccinee had evidence of antibody rise attributed to exposure to wild-type rubella virus at home. Furthermore, Dr. Charles Lebaron reported the results of a more recent vaccine clinical trial designed to evaluate antibody response to a second dose of MMR® given subcutaneously (20). He found that 41% (241/594) of children had four-fold or greater rises in rubella antibody after a second dose of MMR® and noted that these booster responses were restricted to children with less than 50 rubella International Units of antibody per mL. Vaccinees with high levels of rubella antibody (i.e., greater than 50 IU/mL) did not exhibit any additional rise in antibody levels after their second dose because they already had sufficient antibody to neutralize the challenge virus, not because they were tolerant.

**FDA Cited References:**

3. Hayflick, J., International Association of Microbiological Society, Cell Culture Committee of the Permanent Section on Microbiological Standardization; minutes of the Seventh Meeting, held at the Institute of Hygiene, Geneva Switzerland, September 14, 1970.


17. Personal communication to CBER, February 2003.


COMMENT: Many of the citations provided by the FDA related to the safety of the rubella vaccine are from studies conducted more than 30 years ago. It is important to note that 30 years ago there was no PCR testing. That advance came in 1985, and PCR has revolutionized modern medicine enabling advancements in molecular genetics and the study of DNA, RNA and other components of human cells. SR-FTIR spectromicroscopy [Synchrotron Radiation-Based Fourier Transform Infrared spectromicroscopy] was introduced in 2000. This technique allows researchers to follow subtle chemical and molecular changes in individual human cells, without killing the cells or using intrusive probes. This technique allows researchers to monitor different chemical reactions and physical changes inside the cell and it can be used to identify and monitor the progress of diseases in human cells.

As a consumer of vaccines I would like to see more up-to-date safety studies done on existing vaccines, and I would like to be reassured by technologies that are available today that 40-year-old vaccines remain safe for consumption by all individuals regardless of sex, race and/or genetic makeup.